UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

[10/11/94]
[TXR # 0050076]

SUBJECT: Carcinogenicity Peer Review of CHLORPROPHAM

FROM: David G. Anderson, Ph.D.
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Health Effects Division (7509C)

and

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TO: Robert Taylor
Product Manager #25
Fungicide and Herbicide Branch
Registration Division (7505C)

and

Walter Waldrop
Product Manager #71
Special Review and Reregistration Division
Reregistration Division (7508W)

THROUGH: Stephanie R. Irene, Ph.D.
Acting Deputy Director
Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on July 20, 1994 to discuss and evaluate the weight-of-the-evidence on chlorpropham with particular reference to its carcinogenic potential. The CPRC concluded that chlorpropham should be classified as Group E.

SUMMARY

Administration of chlorpropham in the diet to Sprague-Dawley rats was associated with a statistically significant increase in testicular interstitial cell tumors (benign) at the highest dose (which was equal to the limit dose). The increase in testicular tumors was statistically significant by pairwise comparison with controls, exceeded the incidence in historical controls and there was a statistically significant trend.

Significant increases in testicular tumors in the rat occurred only at the highest dose in this study (there was a significant increase at the lowest dose also, which the CPRC considered to be spurious, since it was not maintained at the intermediate doses). The CPRC considered the highest dose to be excessive, based on body weight gain decrements >10% observed during the study in both sexes. There were also other signs of toxicity. [Details are provided in Section F. "The Weight of Evidence".]

There was limited evidence of genotoxicity for chlorpropham: chromosomal aberrations in CHO cells (presumptively positive at moderately toxic doses, with S9 activation only) and dose related transformation in *in vitro* SHE cells, but negative for gene mutation in mouse lymphoma cells. The 3-chloroaniline metabolite of chlorpropham is an analog of a known carcinogen (4-chloroaniline). Structural analogs of chlorpropham: vinclozolin, procymidone and linuron, are also associated with testicular interstitial cell tumors in rats.

In the mouse, at adequate doses, there were no statistically significant increases in the incidence of neoplasia reported in any tissue/organ.

A. Individuals in Attendance at the meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene ____________________

Reto Engler ____________________

William Burnam ____________________

Karl Baetcke ____________________

Marcia Van Gemert ____________________

Elizabeth Doyle ____________________

Hugh Pettigrew ____________________

Esther Rinde ____________________

Richard Hill ____________________

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

David Anderson[1] ____________________

Lori Brunsman ____________________

3. Other Attendees:

Bernice Fisher and Ann Clevenger (HED)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

B. Material Reviewed

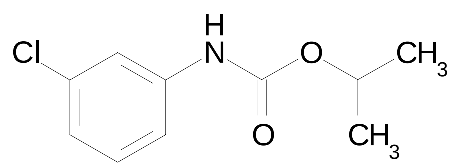
The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Anderson, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information:

Chlorpropham is a growth regulator currently being supported for reregistration only for spinach, controlling sprouting in stored potatoes and for use on Ester lily, St. Augustinegrass, Zoysiagrass, Ginko and for formulation of products. The chemical name is isopropyl-m-chlorocarbanilate, synonyms are CIPC.

LIST A
Cas number: 101-21-3.
Case Number: 818637 and 051517.
Chemical Number: 018301.
Chemical Name: Chlorpropham, CICP; isopropyl-m-chlorocarbanilate.
Vapor Pressure: $1X10^{-5}$ torr.
Water solubility: 88 mg/l.
Tolerances in stored potatoes: 50 ppm.
Tolerances in spinach: 0.3 ppm.
Tolerances in eggs, milk and meat: 0.05 ppm.

Fig. 1:Chlorpropham



D. Evaluation of Carcinogenicity Evidence:

1. Sprague Dawley Rat Carcinogenicity Study

Reference: Botta, JA: "24-Month Combined Oncogenicity/Toxicity Evaluation of Chlorpropham in the Rat", April 22, 1993. MRID# 427547-01. Study# 393L-103-055-89 conducted by T.P.S., Inc., Mt. Vernon, IN, for the Chlorpropham Task Force, John Wise and Associates, Ltd., Liberty, MO.

a. <u>Experimental Design</u>

Chlorpropham was administered in the feed and adjusted to a constant dose level in mg/kg/day to 50 Sprague Dawley rats per sex per group at 0, 30, 100, 500 or 1000 mg/kg/day for 2-years and 10 rats per sex per group for 53-weeks, interim sacrifice.

Body weights, necropsy, organ weight and histological data were recorded for the interim and terminal sacrificed animals. All animals were examined clinically, necropsied and examined histologically. Food and water were supplied ad libitum.

b. <u>Discussion of Tumor Data</u>

The only test material related neoplastic lesions seen with chlorpropham in this study were benign testicular Leydig Cell tumors and focal cell hyperplasia of the testicular Leydig Cells at the highest dose level tested in rats.

Male rats had a significant increasing trend, in addition to a significant difference in the pair-wise comparison of the 1000 mg/kg/day dose group with the controls, for testicular Leydig cell benign tumors, both at $p < 0.01$ (Table 1). There was also a significant difference in the pair-wise comparison of the 30 mg/kg/day dose group with the controls for testicular Leydig cell benign tumors at $p < 0.05$ (Table 1).

There were no significant test material related tumors observed in female rats.

These statistical analyses were based upon Peto's prevalence test since there was a statistically significant negative trend for mortality in male rats with increasing doses of Chlorpropham.

The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of Chlorpropham in both male and female rats. Female rats also showed significant differences in the pair-wise comparisons of mortality of the controls with the 30, 500, and 1000 mg/kg/day dose groups.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

No Leydig Cell tumors or focal cell hyperplasia were seen in animals from the interim sacrifice and only 1, 1, 0 and 4 tumors were seen in dead or sacrificed moribund animals at 0, 30, 100, 500 and 1000 mg/kg/day, respectively.

Historical control data from a limited number of animals (100) indicated a 4% incidence and an incidence in combined controls and negative dosed groups[2] of 6.7% for testicular Leydig cell tumors (benign or malignant not specified) and hyperplasia (Table 1). Historical control data was submitted by the testing laboratory from 2 studies and from Charles River, the animal supplier. The 2 studies from the testing laboratory indicated a testicular Leydig cell tumor incidence of 16 of 350 animals (4.6%) with a range of 3.0% to 6.7% (Tables 2 and 3). Data from Charles River indicated 55 of 880 animals (6.3%, range 0-12%) (Tables 2 and 3). The HDT testicular Leydig cell tumor incidence is 9 of 40 animals (22%),
but the overall testicular Leydig cell tumor incidence in controls and dosed groups from the submitted study was 20 of 297 (6.7%)(Tables 2 and 3).

Body weights for males were 81%** and 75%** of controls at 500 and 1000 mg/kg/day, respectively, and for females they were 77%** and 71% ** of controls for the same respective dose level (** = $p \leq 0.01$). Weekly food consumption was periodically significantly increased and decreased, but mean food consumption for the entire study was increased at the 500 and 1000 mg/kg/day dose level.

Table 1: Chlorpropham - Charles River Sprague-Dawley Rat Study. Male Testis Interstitial Cell (Leydig Cell) Tumor Rates[+] and Peto Prevalence Test Results (p values).

Dose (mg/kg/day)	Control	30	100	500	1000
Benign Tumor incidence (%)	1/33 (3)	4/37 (11)	2/33 (6)	4/40 (10)	9[a]/40 (22)
p =	0.007**	0.018*	0.167	0.112	0.006**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a] First benign tumor observed at week 81, dose 1000 mg/kg/day.

Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

Note:Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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[2] Since the testing laboratory had conducted only 2 other carcinogenicity studies with Charles River CD Sprague Dawley rats, controls and dosed groups where no significant increase in testicular Leydig Cell tumors occurred were combined to increase the number of animals included in the historical control data base. These dosed groups were called "negative dosed groups." Table 3 indicates the percentages from each type of historical control groups, control groups and negative dosed groups.

Table 2: Summary historical control data from 2 studies (controls and negative dosed groups combined) conducted in the testing laboratory, from Charles River and from the current study.

Data source	Number of animals	Total number of Leydig Cell tumors	Percentage of animals
T.P.S. Study No: 192A-101-050-83	150	10	6.7%
T.P.S. study no.: 376A-101-650-89	200	6	3.0%
Charles River	880	55	6.3%
T.P.S. study no.: 393L-103-055-89 (current study)	297	20	6.7%

Table 3: Historical control data individual control and negative dosed groups.

Data source	Controls	Dose group 1	Dose group 2	Dose group 3
T.P.S. Study No: 192A-101-050-83	(50)	(50)	(50)	-
-Focal Leydig Cell hyperplasia	1 (2%)	3 (6%)	2 (4%)	-
-Leydig Cell tumor	0 (0%)	5 (10%)	5 (10%)	-
T.P.S. study no.: 376A-101-650-89	(50)	(50)	(50)	(50)
-Focal Leydig Cell hyperplasia	1 (2%)	0 (0%)	1 (2%)	0 (0%)
-Leydig Cell tumor	2 (4%)	0 (0%)	2 (4%)	2 (4%)
Average % Leydig Cell tumors from historical control data	2/100 (2%)			
Average % Leydig Cell tumors from historical controls and dose groups	16/350 (4.6%)			

Charles River	(880)	Range
-Leydig Cell tumor (B)	31 (3.5%)	0-12.0%
-Leydig Cell tumor (M)	1 (0.1%)	0-1.1%
-Leydig Cell tumor (NOS)	23 (2.6%)	0-9.1%

(50), (880), (%), (B), (M), (NOS) = (Total of 50 or 880 males studied), (percentage), Benign, malignant and not otherwise specified, respectively.

c. Non-neoplastic Lesions

The hematological findings in rats from exposure to chlorpropham are consistent with methemoglobin formation and compensated destruction of red blood cells. At the 100 mg/kg dose level in females, there was increase in hematopoiesis (primarily erythropoiesis) and a decrease in red blood cell count in both sexes at 26 and 53 weeks and an increase in splenic hemosiderosis in females. In addition, at the two highest dose levels there was an increase in the incidence and severity of hematopoiesis in the liver, spleen and bone marrow; an increased incidence of hemosiderosis in the spleen in both sexes; and an increase pigment accumulation in the liver and the kidney tubules of both sexes. Also at the 500 and 1000 mg/kg/day dose levels, there was a significant decrease in red blood cell parameters (RBC, HCT and HGB) and an increase in reticulocyte counts at most intervals of analysis as well as urinary bilirubin (data not shown).

A statistically significant decrease in body weight and body weight gain was also observed at the 500 and 1000 mg/kg/day dose levels.

Although, no definitive antiandrogenic activity was seen in animals treated with chlorpropham, seminal vesicle atrophy (14%) was seen in the HDT animals and only 5% in control animals. A metabolite of chlorpropham is structurally related to two antiandrogenic metabolites, M2 and hydroxyflutamide (See the section on structure activity).

e. <u>Adequacy of the dosing Assessment of the Carcinogenic Potential</u>

The adequacy of the dosing in the 24-month study in Sprague Dawley rats dosed at 0, 30, 100, 500 and 1000 mg/kg/day is indicated by the compensated RBC destruction (Tables 6, 7 and 8) at 100, 500 and 1000 mg/kg/day and the statistically significant lower body weight, the lower body weight gain than in controls and the decreased relative efficiency of food utilization in males and females at the two highest dose levels. The body weight changes in the males and female at 500 mg/kg/day indicate that this dose level is adequate to assess the carcinogenic potential of chlorpropham; the 1000 mg/kg/day dose level was possibly excessive.

At the 500 and 1000 mg/kg/day dose levels, the male body weight was 81% and 75% ($p \leq 0.01$) of controls and body weight gain was 75% and 65% ($p \leq 0.01$) of controls, respectively, at week 104 (Table 7). At 100, 500 and 1000 mg/kg/day, the female body weight gain was 79% ($p \leq 0.05$), 77% and 75% (both $p \leq 0.01$) of controls and female body weight gain was 70%, 67% and 60% (all, $p \leq 0.01$) of controls, respectively, at termination, week 100. Even at week 52, both body weights and body weight gain were statistically significantly reduced in both ($p \leq 0.01$) males and females at the two highest dose levels.

Food consumption was statistically significantly ($p \leq 0.01$) increased in males frequently at 500 mg/kg/day and throughout the study at 1000 mg/kg/day. Food consumption in females was statistically significantly increased infrequently at 500 mg/kg/day and frequently at 1000 mg/kg/day. Relative efficiency of food utilizations were 98%, 89%, 70% and 56% in males and 100%, 92%, 71% and 59% of controls in females, respectively, at the 30, 100, 500 and 1000 mg/kg/day dose levels.

2. CD-1 Mouse Carcinogenicity Study

Reference: Botta, JA: "18-Month Oncogenicity Evaluation of Chlorpropham in the Mouse.", October 21, 1992. MRID# 425303-01. Study# 393K-002-050-89 conducted by T.P.S., Inc., Mt. Vernon, IN, for the Chlorpropham Task Force, John Wise and Associates, Ltd., Liberty, MO.

a. Experimental Design

Chlorpropham was administered in the feed and adjusted to a constant dose level in mg/kg/day to 50 CD-1 mice per sex per group at 0, 100, 500 or 1000 mg/kg/day for the 78-weeks sacrifice and 10 mice per sex per group for the 52-weeks sacrifice.

Body weights, necropsy, organ weight and histological data were recorded for the interim sacrificed and terminal animals. All animals were examined clinically, necropsied and examined histologically. Food and water were supplied ad libitum.

b. Discussion of Tumor Data

No dose related tumors were reported. No statistically significant increases in the incidence of neoplasia in any tissue/organ were observed in either sex when compared with concurrent control values. Total tumor count (all tumor types in all organs/tissue, benign and malignant) were nominally increased in females 16 at 1000 mg/kg/day versus 14 in controls, but none were statistically significant.

The incidence of all neoplasia were within the normal range for 18-month studies in the Charles River CD-1 (ICR) mice (Data submitted to Clement International Corp.: Lang, P., Charles River Laboratories, 1991 and data submitted to T.P.S Laboratories from Charles River, attachment). This was the first mouse carcinogenicity study conducted at the testing laboratory in the Charles River CD-1 mouse, thus historical control data from the animal supplier were the only data available other than the concurrent control data.

c. Non-neoplastic Lesions

The hematological findings in mice from exposure to chlorpropham are consistent with methemoglobin formation and compensated destruction of red blood cells. At the 500 mg/kg/day dose level males and females demonstrated increased hemosiderosis in the spleen in the interim and terminally sacrificed animals (about 50% males and females at 500 mg/kg/day and about 70-90% males and females at 1000 mg/kg/day) and increased hematopoiesis in spleen, liver and bone marrow in males and females. Slight hematopoiesis (Under the laboratory definition slight hematopoiesis is normal.) was found in the spleens of 12%, 14%, 36% and 60% in males at 0, 100, 500 and 1000 mg/kg/day dose levels, respectively. Moderate or marked hematopoiesis were found in 2%, 0%, 8% and 18% at the same respective dose levels. Slight hematopoiesis was found in the spleens of females at 30%, 30%, 52% and 70% at the same respective dose levels and marked or moderate hematopoiesis was found at 8%, 0%, 6% and 14% at the same respective dose levels. Remarkably dark eyes and bluish extremities were noted and were consistent with possible methemoglobinemia.

In addition, at 1000 mg/kg/day the percentage of reticulocyte for males (1.1 in controls versus 4.2 at 1000 mg/kg/day, $p \le 0.05$) was increased, but not for females. By termination animals appeared to recover slightly because reticulocyte counts were higher at the 52 week sacrifice than at term. In the interim sacrifice, reticulocyte counts in males were 1.8 in controls versus 3.1, $p \le 0.01$ at 500 mg/kg/day, but in females they were statistically significantly increased only at the 1000 mg/kg/day dose level. Increases occurred in MCH and MCHC for males and females. Decreased survival occurred in males (58% versus 86% in controls).

Increased absolute and relative spleen (136% and 139% of controls, respectively, $p \le 0.01$) and absolute and relative liver weight (111%, $p > 0.05$, and 108%, $p < 0.05$), respectively) were noted in males at 1000 mg/kg/day at termination. Female spleen and liver weight followed similar patterns, but only relative spleen weights were statistically significantly increased. Ovarian weights were 38% of control values at terminal sacrifice of the 1000 mg/kg/day dosed females.

Amyloidosis was treatment related at the 1000 mg/kg/day dose level and it was the principle cause of death in the males.

The NOEL/LEL are 100 mg/kg/day and 500 mg/kg/day for hematological increased hematopoiesis and reticulocyte, probably due to increased destruction of red blood cells from methemoglobin formation.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Chlorpropham was administered to both sexes in mice at greater than the limit dose level at the highest dose tested. Since no increased tumor incidence occurred over concurrent controls or historical controls at this limit dose level, higher dose levels need not be considered. The dose levels were adequate to assess the carcinogenic potential of chlorpropham in the mouse.

The mean chlorpropham consumption over the course of the study at the nominal dose levels of 100, 500 or 1000 mg/kg/day were 108.9 ± 9.2, 523.0 ± 42.0 and 1050.6 ± 82.8 mg/kg/day, respectively. No consistent dose related body weight or body weight gains were observed.

Mortality was determined at week 13, 26, 52, 65 and 78. Statistically significant increased mortality occurred only in males (58%) at the 1000 mg/kg/day dose level compared with control values (86%) at week 78. In terminal females at the same dose level, there was a nominal increase in mortality (78% versus 84% in controls). The major cause of death was reported to be amyloidosis. The Charles River CD-1 mouse typically has a high background for amyloidosis.

E. Additional Toxicity Data on Chlorpropham:

1. <u>Metabolism</u>

> Robinson, RA and Liu, David DW, Metabolism of 14C-Chlorpropham in Rats - Definitive FIFRA study, Metabolism Analysis and Quantitation. August 20, 1991. Study No. XBL90051. Report No. RPT0058. MRID# 420069-01.

Chlorpropham was administered by gavage to 5 Sprague Dawley rats per sex per group at 5 mg/kg and 200 mg/kg/day in single doses or multiple doses over 15 days, respectively. A single 0.5 mg/kg i.v. dose was also administered to other groups of rats.

Chlorpropham was rapidly absorbed and metabolized essentially 100% prior to excretion in the urine with small amounts in feces. Within 24 hours 82-92% of the dose was recovered in the urine and 3-5% in the feces. Peak excretion at the low dose occurred at 4-12 hours (49-62% of the dose) and at the high dose between 8-24 hours (59-64% of the dose). Less than 0.03% of doses were recovered as $[c^{14}]CO_2$ over a 3-day period. The approximate one-half life of chlorpropham in the rat is 8 hours at the low dose and 9 hours for the high dose in males and females. Three major metabolic routes are proposed for chlorpropham; (1) hydroxylation at the 4'-position and conjugation, (2) oxidation of the isopropyl side chain to form isopropanol and isopropinate moieties and (3) decarbanilation to form 3-chloroaniline, then N-acetylation.

2. <u>Mutagenicity</u>

Chlorpropham treatment resulted in chromosomal aberrations in CHO cells and cell transformation in SHE cells in two adequately conducted assays, but it was not mutagenic in a mouse lymphoma assay. Two other supplementary assays were inconclusive. Two adequate Ames assays of two chlorpropham potential metabolites (isopropyl-5-chloro-2-hydroxy carbanilate, PPG-134, isopropyl-3-chloro-4-hydroxy carbanilate, PPG 154) and were negative. The Toxicity Chapter for the Chlorpropham Registration Standard referenced a large variety of positive and negative mutagenicity assays in the literature, but the information was not considered adequate for risk assessment.

a) Mouse Lymphoma (L5178Y/TK+/-) Assay for Mutation - Not mutagenic at dose levels of 0.01 - 10,000 μg/ml. 13 - 100 μg/ml. Toxicity noted at 1000 - 10,000 μg/ml. Accession 250808; MRID# 00129938.

b) Chromosomal Aberration in CHO Cells - Presumptively positive at moderately toxic doses of 120 and 140 μg/ml, but only in the presence of S9. Negative in absence of S9. Accession/MRID# 418462-01.

c) In vitro SHE Cell Transformation - Dose related stable morphological transformation. The transformation occurred at relatively non-toxic doses exposed under two different regimens. MRID# 418455-01.

d) Ames Assay - Inconclusive and supplementary.

e) Cell Transformation in BALB/C3T3 cells - Inconclusive and supplementary.

d) A large (>100) variety of positive and negative mutagenicity studies in the literature. Considered inadequate and referenced in HED Doc# 007767, The Toxicity Chapter of the Chlorpropham Registration Standard.

Metabolites:

a) Salmonella Assay - PPG-134 (isopropyl-5-chloro-2-hydroxy carbanilate) was not mutagenic in TA98, TA100, TA1535, TA1437 and TA1538 strains with and without S9. Accession/MRID# 249883.

b) Salmonella Assay (Ames) - PPG-154 (isopropyl-3-chloro-4-hydroxycarbanilate) was not mutagenic in TA98, TA100, TA1535, TA1437 and TA1538 strains with and without S9. Accession/MRID# 249883.

3. <u>Structure-Activity Correlations</u>

Several analogs of chlorpropham (Figure 2) were proposed; some are not adequately tested for a determination and some structures may not be adequately analogous. Propham and fluometuron appear to be negative for carcinogenicity, but the data have not been reviewed by the RfD Committee or the CPRC. Procymidone and iprodione have been classified as carcinogens by the CPRC; diuron also appears to be carcingenic, but the data have not been reviewed by the CPRC. Vinclozolin, procymidone, iprodione and also linuron are among the pesticides that have been associated with testicular Leydig Cell hyperplasia and tumors in rats.

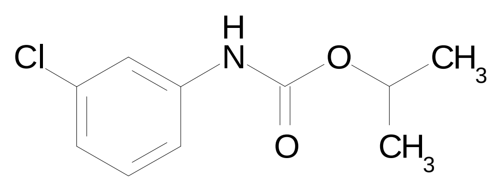

Figure 2: Chlorpropham

Propham (Figure 3) is structurally related (it lacks a chloro group on the ring), but there is no mutagenicity or acceptable chronic studies for this pesticide. An unacceptable 2-year rat study (reviewed in 1967) at 45, 135 and 450 mg/kg/day indicated that random subcutaneous fibromas or adenomas occurred during the study but that test animals did not differ from controls other than a "mild reduction in weight gain". The data on propham have not been reviewed by the CPRC.

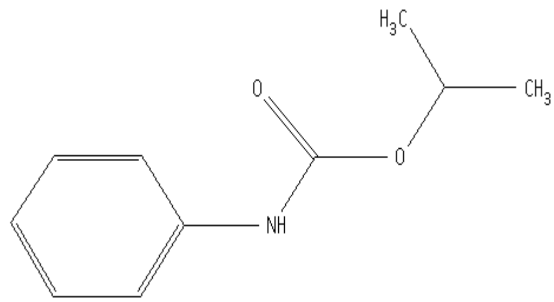

Figure 3: Propham

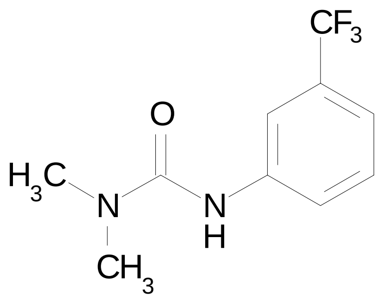

Figure 4:Fluometuron

Fluometuron (Figure 4) differs from chlorpropham by a substitution of meta trifluoro group for the meta chloro group on the phenyl ring and the dimethyl urea side chain. Fluometuron is negative for carcinogenicity in the rat and mouse according to the reviewer of the studies. Only the rat study was adequate. Fluometuron was negative in an Ames test, Micronucleus test and for Unscheduled DNA synthesis. The data on fluometron have not been reviewed by the RfD Committee or the CPRC.

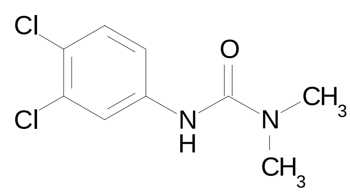

Figure 5: Diuron

Diuron (Figure 5) differs from chlorpropham by a 3,4-dichloro substituted instead of a 3-chloro substituted phenyl ring and the dimethylurea side chain. As stated by the reviewer of the studies, both the rat and mouse studies are supplementary with the rat study showing an increase urinary bladder and renal pelvis epithelial papillomas and carcinomas at 250 ppm (HDT) and the mouse study showing increases in ovarian luteomas and mammary gland adenomas. Diuron was clastogenic in an in vitro cytogenic assay, but negative for unscheduled DNA synthesis, in a HGPRT (CHO) assay and an Ames (S. typh.) assay, all acceptable. The data on diuron have not been reviewed by the CPRC.

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Cl
 \ _
 /   \
   o    - NH      OH
 \ _ /     \    /
 /         //‾ C - CH=CH2
Cl         O    \
                CH3
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Irreversible degradation product, referred to as Compound 23 {3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide} in the metabolism study and M2 by Szeto et al., 1989.

Figure 6: M2

M2 is probably the most active antiandrogenic metabolite M2 (Figure 6) of Vinclozolin and is a close analog of chlorpropham. M2 has not been directly tested for mutagenicity or carcinogenicity.

Vinclozolin (Figure 7) results in testicular benign Leydig Cell, liver and ovarian tumors in the rats in recently submitted studies. A chronic study and a oncogenicity study in rats showed liver carcinomas only at dose levels causing severe weight depression. Only dose levels causing statistical significant death in mice resulted in liver carcinomas. Vinclozolin results in no mutagenic activity in the battery of tests that were at least core minimum. Vinclozolin (Ronilan) is currently classified as a group E carcinogen (based on older studies); however, the recently submitted carcinogenicity studies in the rat and mouse have not been evaluated nor classified by the CPRC.

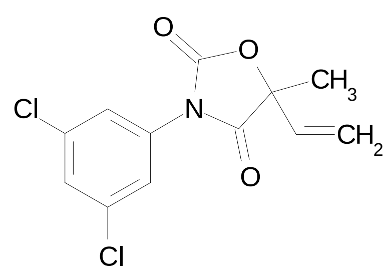

Figure 7:Vinclozolin

Procymidone (Figure 8) differs from chlorpropham in several ways as can be seen in figure 7. Procymidone causes testicular Leydig Cell and liver tumors in rats, pituitary tumors in female rats, and liver tumors in female mice. While Procymidone was negative in a battery of mutagenicity tests, deficiencies in some of the studies were noted. The SAP classified procymidone as a Group C carcinogen; however, procymidone was classified by the CPRC as a Group B2 carcinogen.

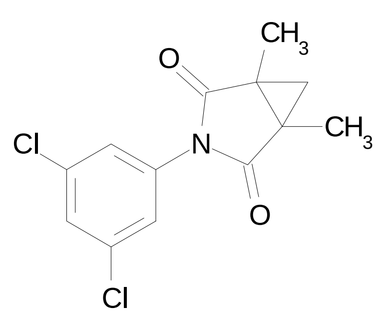

Figure 8:Procymidone

Iprodione (Figure 9) differs from chlorpropham in several ways as can be seen in figure 9. Iprodione causes testicular Leydig Cell tumors in rats and testicular Leydig Cell and liver tumors in mice. Iprodione was positive for DNA repair in B. subtils., but negative in the Ames test, SCE test in CHO cells, forward mutation Mammalian cells, chromosomal aberrations in CHO cells. Iprodione was classified by the CPRC as a Group B2 carcinogen.

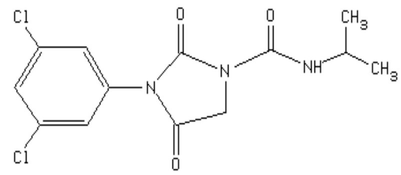

Figure 9: Iprodione

Linuron (Figure 10) differs from chlorpropham by the substituent on the carbanilate group and a 3,4-dichlorophenyl rather than a 3-chlorophenyl group and 1-methyl-1-oxymethyl urea side chain. Linuron causes Leydig cell adenomas in rats and hepatocellular adenomas in mice. Increased RBC destruction occurred in both studies which were acceptable. Linuron was negative in for gene mutation in CHO cells in the Ames test, for chromosomal aberrations and for unscheduled DNA synthesis. Linuron was classified by the CPRC as a Group C carcinogen with no Q*.

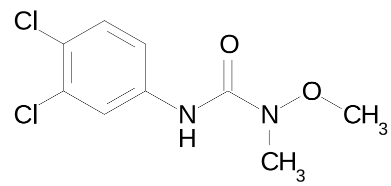

Figure 10: Linuron

Hydroxyflutamide, a metabolite of Flutamide (Figure 11) is a close analog of chlorpropham, differing by only the phenyl ring substituents and the isobutanoate side chain. Flutamide is associated with testicular Leydig Cell tumors in rats and is used to treat benign prostate hypertrophy and prostate cancer in humans [Labrie et al. (1990), Neumann (1991), Pavone-Macaluso et al. (1990), Petrangeli et al. (1988), Rasmussen (1990), Roberts et al. (1989) and The Merck Index (1989)]. The data on flutamide have not been reviewed by the CPRC.

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                                   CH3
             CF3       O          /
             \ _       \\_  CH - CH3
             /   \      /
NO2 -          o    - NH
             \ _ /
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Figure 11: Flutamide

Other structures were similar to chlorpropham, but the data were inadequate to determine the potential for carcinogenicity.

5. Acute, Subchronic and Chronic Studies:

a) Acute Toxicity Studies: (All the acute studies cited below are acceptable.)

The acute oral LD50 in rats for dosed with chlorpropham is 4.2 mg/kg for both sexes (Tox Category III). The acute dermal LD50 in rats is < 5 g/kg (Tox Category IV). The acute inhalation toxicity study was waived because of the physical chemical properties of chlorpropham. Minimal irritation was noted in the primary eye (Tox Category III) and dermal irritation studies (Tox Category IV). Chlorpropham is not a dermal sensitizer in the Guinea Pig.

b) Subchronic Studies:

i) 90-Day Studies in rats tested at a constant 0, 17, 70 or 1200 mg/kg/day indicated a NOEL/LEL of < 17 mg/kg/day for treatment related changes in red blood cell morphology, 6/20 animals with target cells and 9/20 animals with crenated cells. At higher dose levels decreased erythrocyte counts and hemoglobin concentration were noted along with increased hematopoiesis in the spleen, liver and bone marrow, in addition to the dose related increases in aberrant red blood cell morphology.

Core classification: Supplementary. The study is not acceptable under Guideline (82-2) for a study in the mouse because potential methemoglobin levels were not determined.

ii) 90-Day Studies in mice tested at 0, 105, 210, 420 or 840 mg/kg/day indicated that NOEL/LEL of 420 mg/kg/day and 840 mg/kg/day, respectively, for males and females. At the LEL darker than usual blood and eyes were noted. Increased spleen and liver weights and increased extramedullary hematopoiesis in livers and spleens.

Core classification: Supplementary. The study is not acceptable under Guideline (82-2) for a study in the rat because potential methemoglobin levels were not determined.

c) Chronic Studies:

One-Year Study in dogs tested at 0, 5, 50, 350 or 500 mg/kg/day indicated a NOEL/LEL of 5 and 50 mg/kg/day for absolute and relative thyroid weight increases and enlargement irregular shaped follicles lined with medium to high cuboidal epithelium containing clear to pale colloid. T4 and T3 levels were decreased in animals with large thyroids. TSH stimulation tests indicated that chlorpropham administration resulted in reduced stimulation. At higher dose levels decreased erythrocyte counts and hematocrits occurred as well as increased cholesterol levels.
Core classification: Minimum. The study is acceptable under Guideline (83-2) in the dog.

F. Weight of the Evidence Considerations:

1. Chlorpropham administration to Sprague Dawley rats was associated with a statistically significant ($p \leq 0.01$) increase in benign testicular Leydig cell tumors and a statistically significant increased trend ($p \leq 0.01$). The highest dose administered was the limit dose of 1000 mg/kg/day. The tumors at 1000 mg/kg/day were elevated above the historical control data range for the strain of rat used. The statistical significantly increased ($p \leq 0.05$) benign testicular Leydig Cell tumors at the LDT were below the historical control range. Hyperplasia of these cells occurred only in tumor bearing animals. A body weight gain decrement of 35% in males and 40% in females was accompanied by a decreased relative efficiency of food utilization, red blood cell destruction and enlarged spleens at 1000 mg/kg/day. No statistically significant tumors were observed at the 500 mg/kg/day dose level (also with red blood cell destruction and a decrease in body weight and body weight gain), which was adequate to assess the carcinogenic potential of chlorpropham.

2. Chlorpropham administration to CD-1 mice caused a nominal increase of combined benign and malignant neoplasia of various types in females at the limit dose of 1000 mg/kg/day, but no increase in single tissue neoplasia. None of the neoplastic lesions were statistically significant and all tumor incidence was below the historical control data for the CD-1 mouse.

3. Chlorpropham treatment resulted in chromosomal aberrations in CHO cells and cell transformation in SHE cells in two adequately conducted assays, but it was not mutagenic in a mouse lymphoma assay. Two other supplementary assays were inconclusive. Two adequate Ames assays of two chlorpropham metabolites (isopropyl-5-chloro-2-hydroxy carbanilate and isopropyl-3-chloro-4-hydroxy carbanilate) were negative.

4. Three major metabolic routes are proposed for chlorpropham; (1) hydroxylation at the 4'-position and conjugation. (2) oxidation of the isopropyl side chain to from isopropanol and isopropinate moieties, and (3) decarbanilation to form 3-chloroaniline (1-2% of metabolites excreted in urine from a 5 mg/kg dose), then N-acetylation. Two metabolites tested were not mutagenic (PPG 134 - isopropyl-5-chloro-2-hydroxy carbanilate and PPG 154 - isopropyl-3-chloro-4-hydroxy carbanilate).

5. Structural analogs of chlorpropham are also associated with testicular interstitial (Leydig) cell tumors in rats. Some of these pesticides are known to have some antiandrogen component that result in Leydig cell hyperplasia through failure to inhibit excessive release of LH.

6. No antiandrogenic activity has been noted in any of the chlorpropham studies, but sensitive accessory sex organs were not weighed. However, the 1,4'-dihydroxychlorpropham is a relatively close analog to the antiandrogen 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide and hydroxy-flutamide.

7. <u>Carcinogenicity in animals</u> -- Chlorpropham

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to chlorpropham resulted in an increased incidence of testicular interstitial cell adenomas in rats. Although these tumors occurred only at doses considered to be excessively toxic to the rats, the incidence exceeded the historical control range, there was limited evidence of genotoxicity, and structurally analogous chemicals are also associated with testicular interstitial cell tumors. The relevance of the tumor data to an evaluation of chlopropham's potential for human carcinogenicity is discussed elsewhere in this document.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for

Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The consensus of the CPRC was that chlorpropham should be classified as Group E - no evidence for carcinogenicity in humans. This decision was based on the results of two animal studies in different species (mouse and rat). In the mouse, at adequate doses, there were no statistically significant increases in the incidence of neoplasia reported in any tissue/organ. In the rat, although there was a statistically significant increase in tumors (testicular interstitial cell adenomas), it occurred at 1000 mg/kg/day, a dose which the CPRC considered to be excessive. Since there were four doses in this study (instead of the usual three) the CPRC considered the remaining doses: 30, 100 and 500 mg/kg/day to be sufficient for an acceptable cancer study, without considering the results at the 1000 mg/kg/day dose at all.

Although the significant increase in tumors occurred only at an excessive dose, the increase was statistically significant by pairwise comparison with controls ($p<.01$), the incidence was well outside of the historical controls and there was a statistically significant trend ($p<.01$). There was limited evidence of genotoxicity, the 3-chloroaniline metabolite is an analog of a known carcinogen and structurally related chemicals are associated with the same tumor type (testicular interstitial cell) in the rat. While the consensus of the Committee led to a Group E classification, there were some members who felt that the evidence was sufficient to suggest a higher classification.

H. References:

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